# Oligomere

Wintersemester 2011/12

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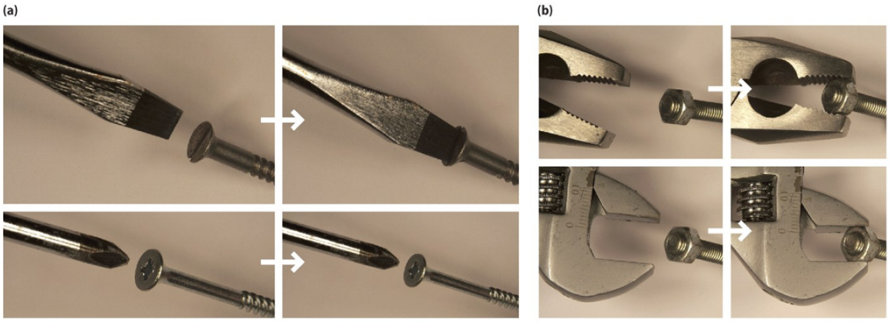
## Oligomers

- Oligomerization is abundant: Approximately two-thirds of human enzymes are oligomers. In *E. Coli*, the average oligomerization state of proteins is 4.
- Proteins can form **homo-oligomers** by self-association, or **hetero-oligomers** by binding to a different protein.
- Homodimers are almost always symmetric.
- Different types of symmetry are possible:
  a) symmetric interactions between monomers: even number of monomers required
  b) cyclic symmetry: any number of monomers possible
  Type (a) occurs about 10 times more often.

*„We may ... ask why molecular evolution should have so frequently favoured the appearance and maintenance of oligomeric globular proteins. That it should be so must mean that there are functional advantages of some kind, inherent in the oligomeric state, and absent or difficult to achieve in the monomeric state.“*

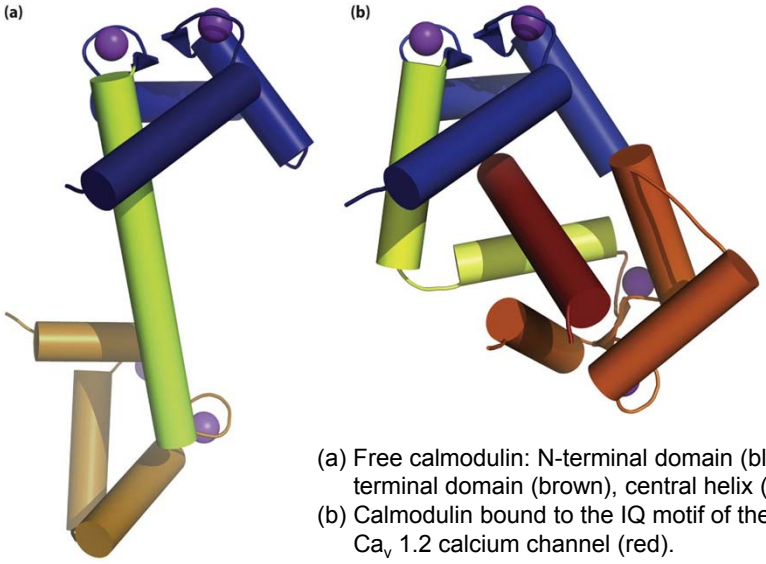
J. Monod, J. Wyman & J.-P. Changeux.
On the nature of allosteric transitions: a plausible model.
J. Mol. Biol. 12, 88–118 (1965).

## Properties of tools



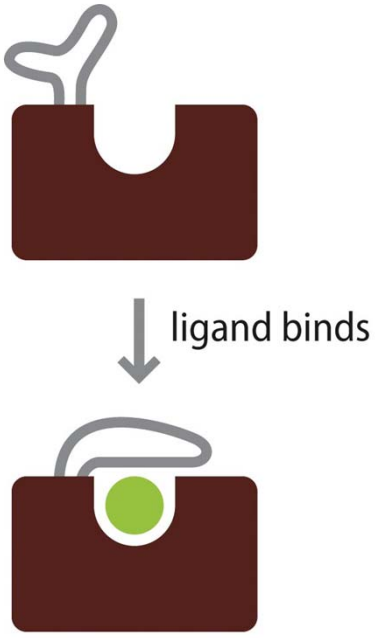

(a) A tool needs to recognize its substrate. Different substrates need different, but related, tools.
(b) A tool needs to change in response to its substrate. The change is often small, but very significant in terms of function.

## Calmodulin structures




(a) Free calmodulin: N-terminal domain (blue), C-terminal domain (brown), central helix (green).
(b) Calmodulin bound to the IQ motif of the cardiac $Ca_v$ 1.2 calcium channel (red).

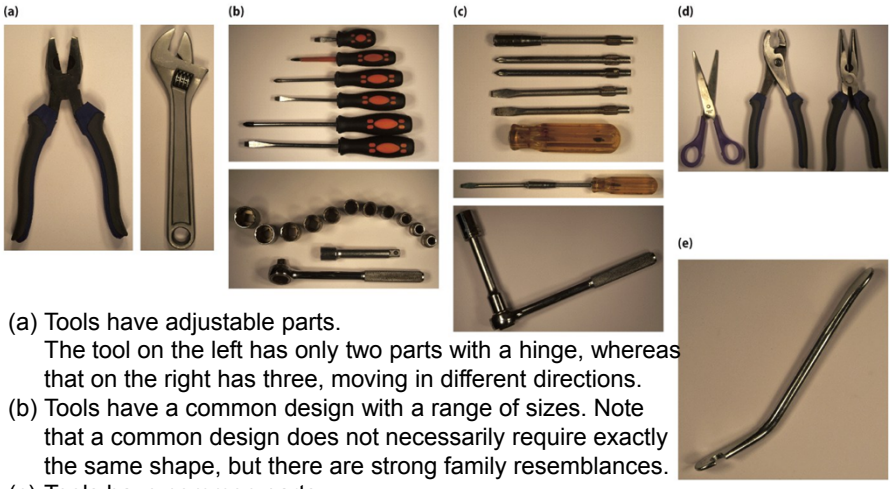
Figure 2.42 How Proteins Work (©2012 Garland Science)




It is very common to find that enzymes contain flaps or lids that are usually disordered in the absence of substrate but close over the bound substrate.

Figure 2.43 How Proteins Work (©2012 Garland Science)

## Design principles of tools




(a) Tools have adjustable parts.
The tool on the left has only two parts with a hinge, whereas that on the right has three, moving in different directions.
(b) Tools have a common design with a range of sizes. Note that a common design does not necessarily require exactly the same shape, but there are strong family resemblances.
(c) Tools have common parts.
(d) Tools have (approximate) symmetry.
(e) A tool can be a specialist, with only a single use.

## Human Pumilio 1 in complex with Puf5 RNA

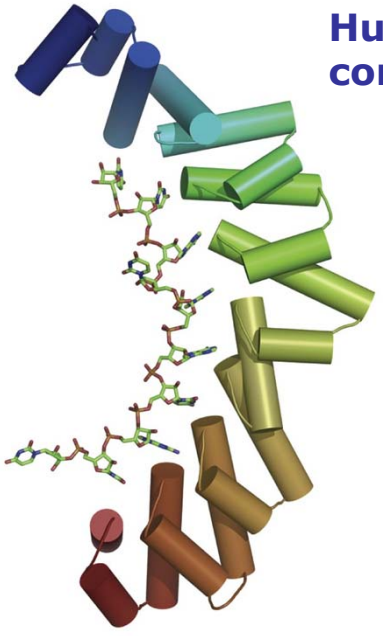


Figure 2.44 How Proteins Work (©2012 Garland Science)

Each pair of helices (except for the first three) recognizes a single base.

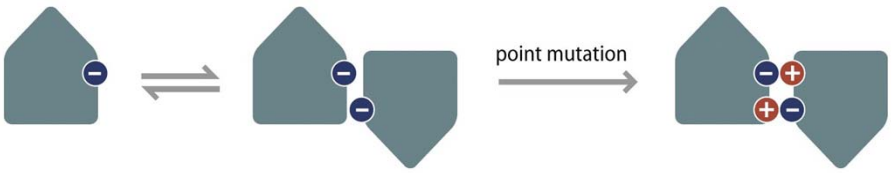



Figure 3.1 How Proteins Work (©2012 Garland Science)

- A monomeric protein with a negative charge on the surface forms a weak dimer.
- A single point mutation, creating a complementary positive charge, generates two symmetrical stabilizing interactions and therefore has a strong influence on the dimerization equilibrium.
- If it is advantageous for the protein to be dimeric, the mutation will quickly be established.

## Amino acid frequencies in oligomeric interfaces and in the protein interior

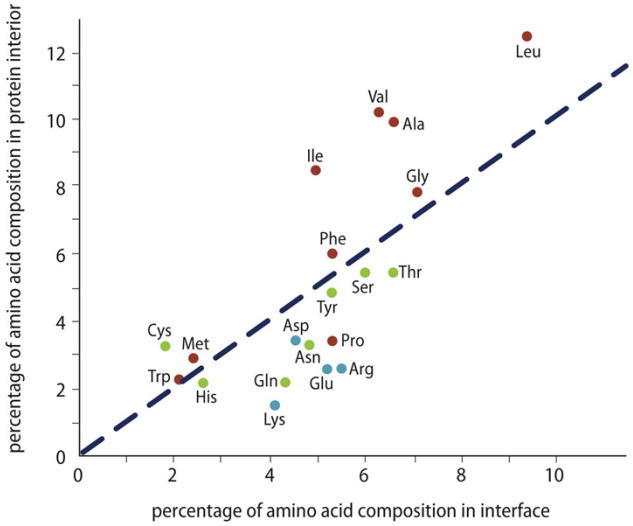



Figure 3.2 How Proteins Work (©2012 Garland Science)

## Regulation of access to the active site by changing the dimer interface

Dimeric single domain enzyme

Dimeric two-domain enzyme

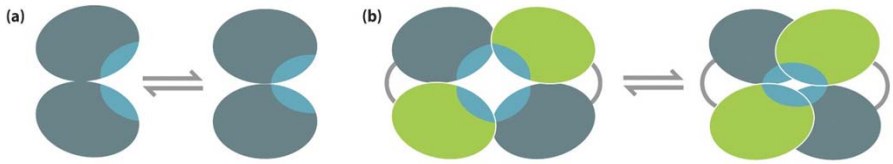



Figure 3.3 How Proteins Work (©2012 Garland Science)

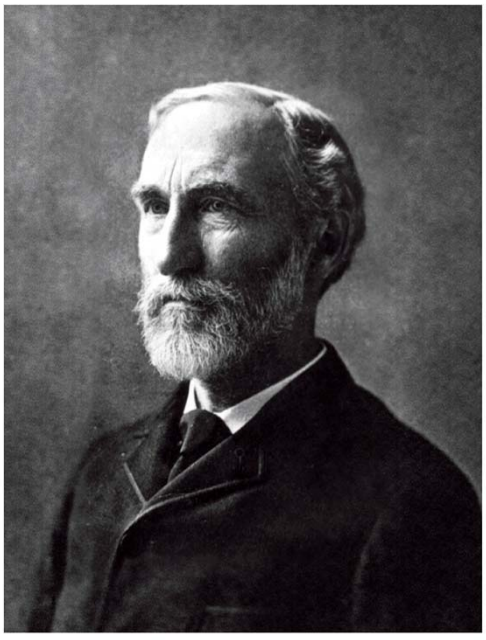
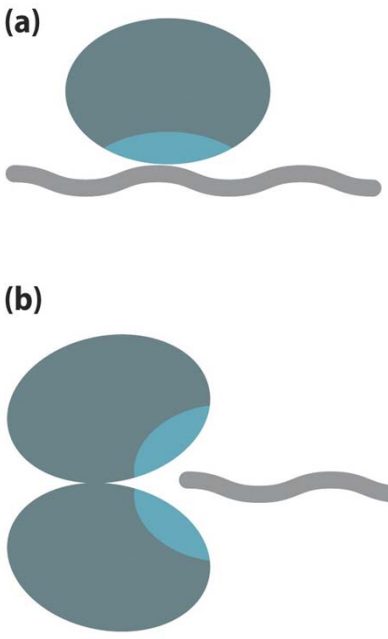


Figure 3.2.1 How Proteins Work (©2012 Garland Science)

$\Delta G = \Delta H - T\,\Delta S$

Josiah Willard Gibbs (1839–1903)




An exposed active site can accommodate a large substrate, whereas a more restrictive site, as in a dimer, can only accommodate a smaller substrate.

Figure 3.4 How Proteins Work (©2012 Garland Science)

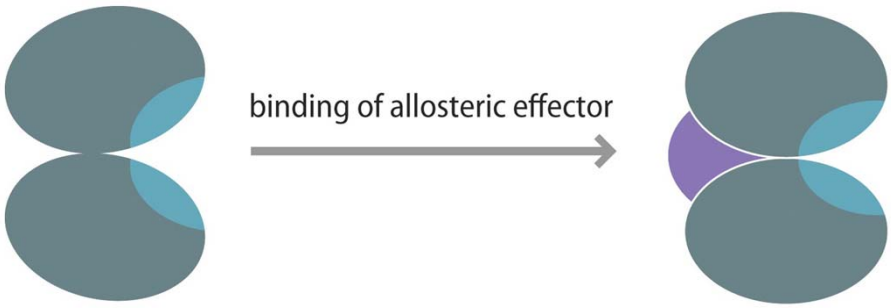



Figure 3.5 How Proteins Work (©2012 Garland Science)

Dimeric enzymes permit the simple evolution of allosteric effectors. Provided that the active site is at the dimer interface, such ligands can bind elsewhere on the interface and can alter its structure, thereby affecting the active site.

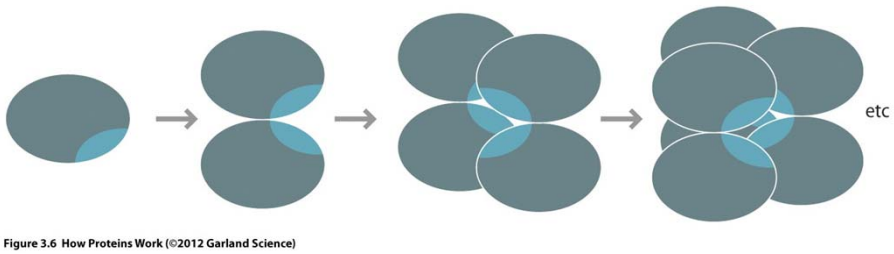
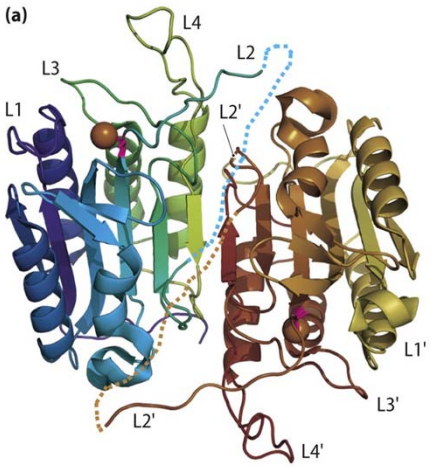



Figure 3.6 How Proteins Work (©2012 Garland Science)

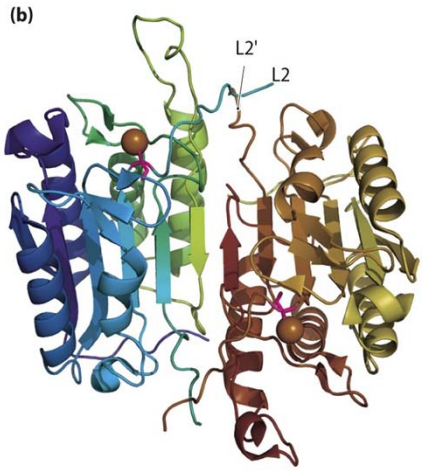
The active site of an enzyme is usually hydrophobic. This can lead to random dimerization and then on to greater degrees of uncontrolled aggregation. A dimer limits the amount of association that can occur.

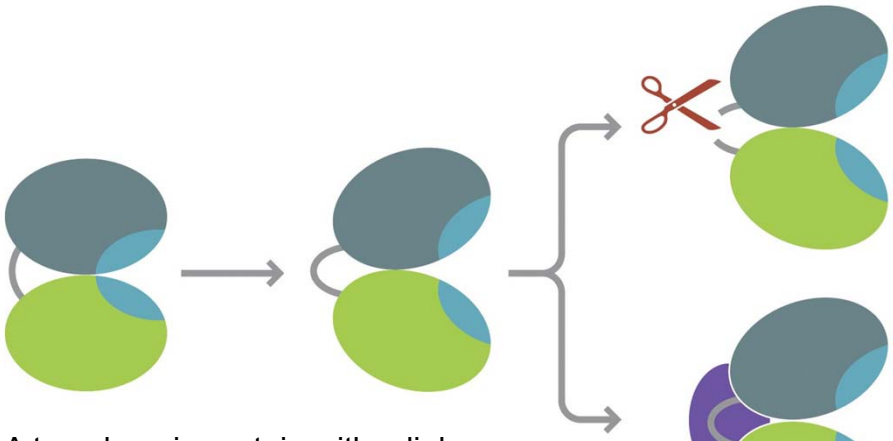
## Activation of caspase-7 by proteolytic cleavage

Uncleaved zymogen




Cleaved active enzyme





A two-domain protein with a linker between the domains has the problem that conformational change of the domain interface leads to loosening of the linker and therefore to a greater potential for degradation or unwanted association of the linker.

## Hepatitis B virus genome structure

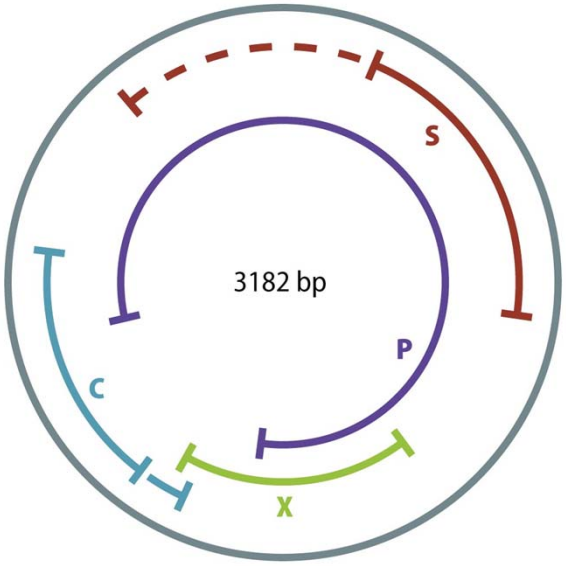



Figure 3.9 How Proteins Work (©2012 Garland Science)

The virus genome codes for only four proteins, two of which have pre-regions (dashed). The overlapping sequences are in different reading frames.

## Hemoglobin $\alpha_2$-$\beta_2$ tetramer

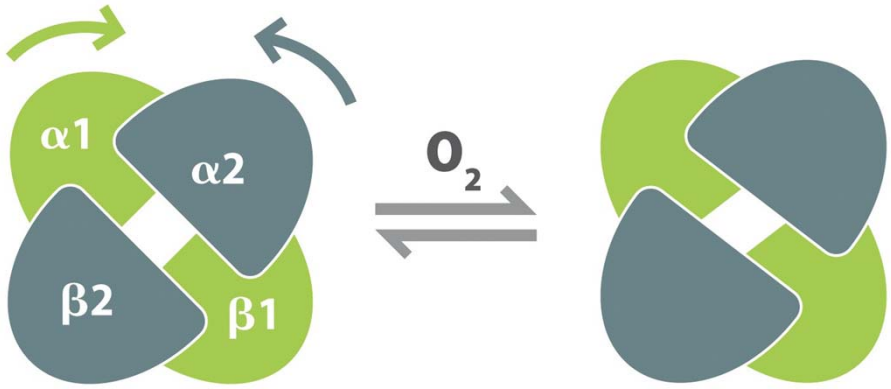



Figure 3.10 How Proteins Work (©2012 Garland Science)

Binding of oxygen leads to the rotation of one αβ pair relative to the other.

## Hemoglobin: Conformational change upon oxidation

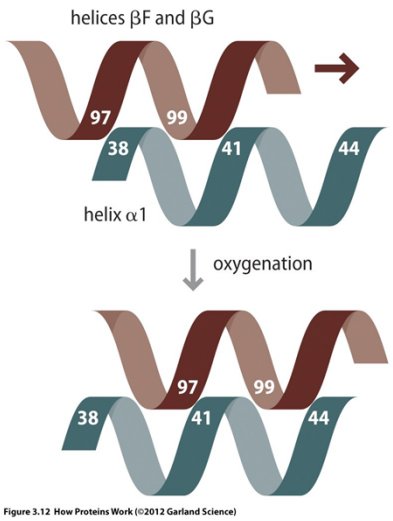
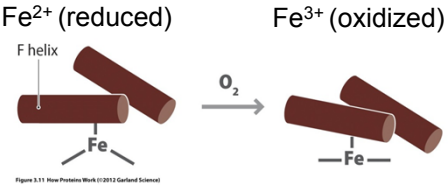



Figure 3.12 How Proteins Work (©2012 Garland Science)




- The interface between helix C on chain $\alpha_1$ and the helix F/G turn on chain $\beta_2$ fits together as knobs into holes in one of two ways.
- Shifting of the F helix moves it out of one arrangement into the other.
- Each arrangement is stabilized by hydrogen bonds.

## Hemoglobin: Thermodynamic cycle

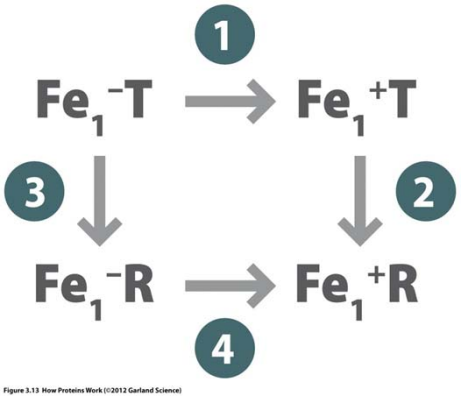



Figure 3.13 How Proteins Work (©2012 Garland Science)

$Fe_1^+$: Oxygen bound to subunit 1
$Fe_1^-$: Oxygen not bound to subunit 1

T: "tense" conformation of interface
R: "relaxed" conformation of interface

## Oxygen saturation curves

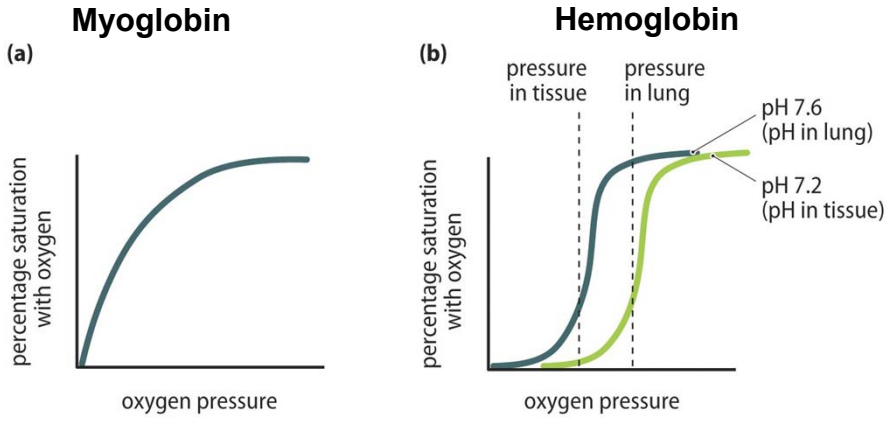



Figure 3.14 How Proteins Work (©2012 Garland Science)

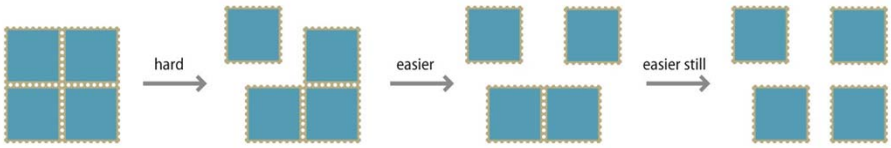



Figure 3.15 How Proteins Work (©2012 Garland Science)

When removing stamps from a block of four, each successive stamp gets progressively less difficult (by a factor of two for each stamp).

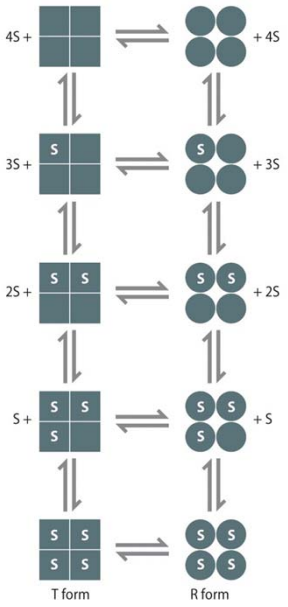
# MWC model for ligand binding to a tetrameric protein

S: substrate

T: “tense” conformation with more interactions than R between subunits. Major form present in the absence of S.

R: “relaxed” conformation with a higher affinity than T for S. In the presence of S the equilibrium shifts to favor the R form.

J. Monod, J. Wyman & J.-P. Changeux.
On the nature of allosteric transitions: a plausible model.
J. Mol. Biol. 12, 88–118 (1965).



## Sequential or KNF model for ligand binding to a tetrameric protein

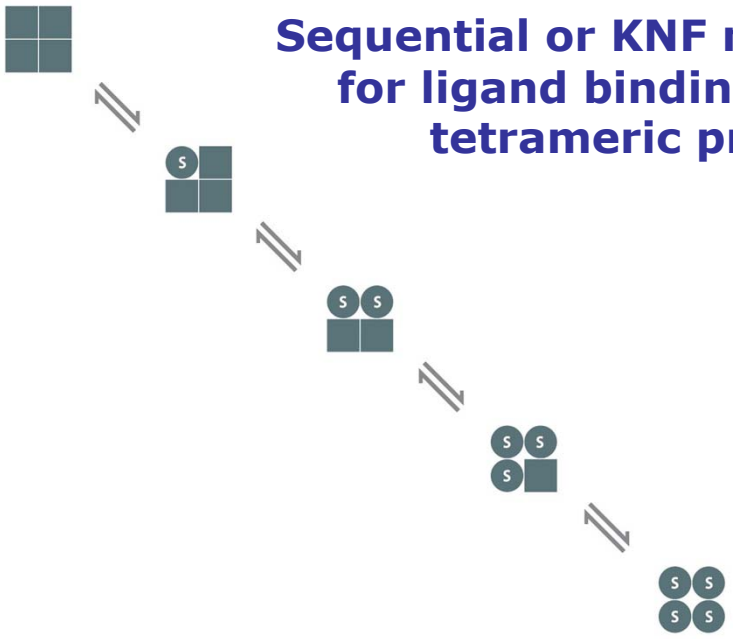


Figure 3.17 How Proteins Work (©2012 Garland Science)

## General scheme for ligand binding to a tetrameric protein

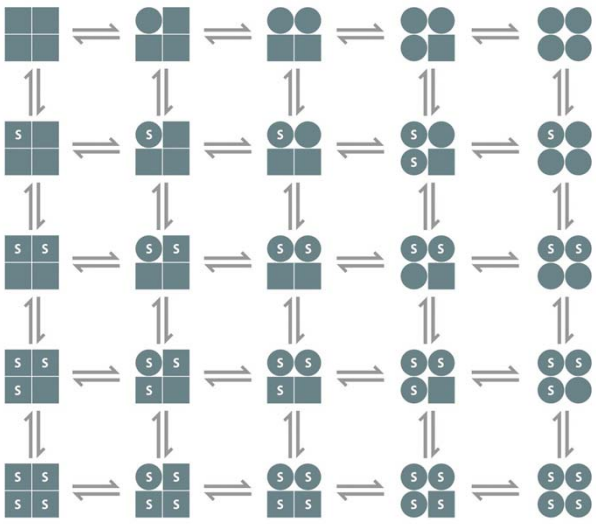


Figure 3.18 How Proteins Work (©2012 Garland Science)

Includes both the MWC model (left and right column) and the KNF (diagonal).

## Phosphorylation of glycogen phosphorylase

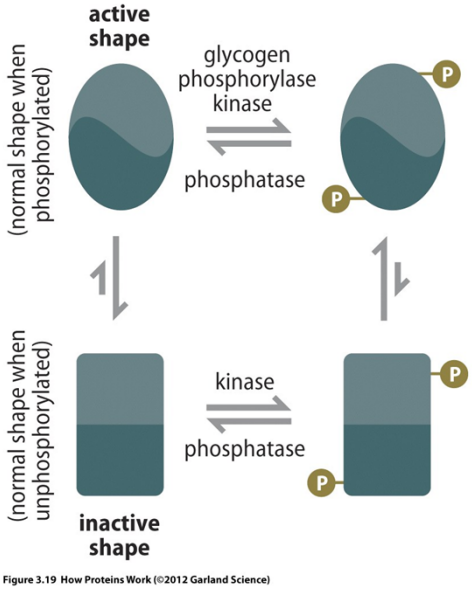
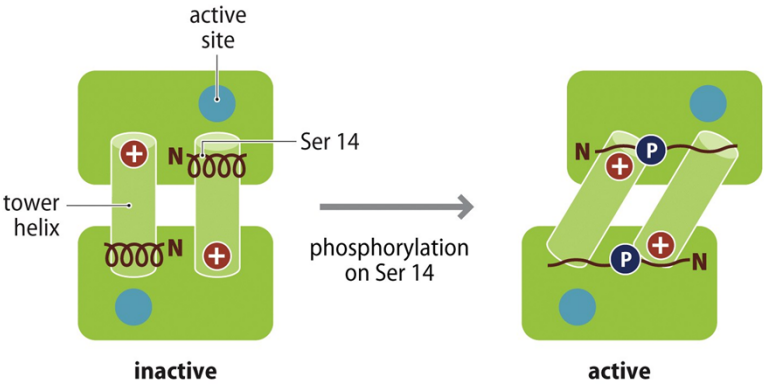



Figure 3.19 How Proteins Work (©2012 Garland Science)

- Phosphorylation alters the equilibrium between two possible conformations and favors the active conformation, thereby markedly increasiung its activity.
- Glycogen phosphorylase is phosphorylated by phosphotylate kinase, which in turn is phosphorylated and activated by cAMP-dependent protein kinase.
- This kinase is activated by an increase in cAMP concentrations, as a consequence of the activation of a receptor triggered by epinephrine (adrenaline).
- Thus, epinephrine leads to an increase in intracellular glucose, in readiness for the "fight or flight" response.

## Mechanism for activation of glycogen phosphorylase by phosphorylation of Ser14




Phosphorylation creates a negative charge, which is attracted to a positive charge on the tower helix of the other monomer. This leads to unwinding of the N-terminal helix and a reaching out of the tower helices. This uncovers the active site and activates the enzyme.

## Allosteric mechanism in a two-domain (but monomeric) enzyme

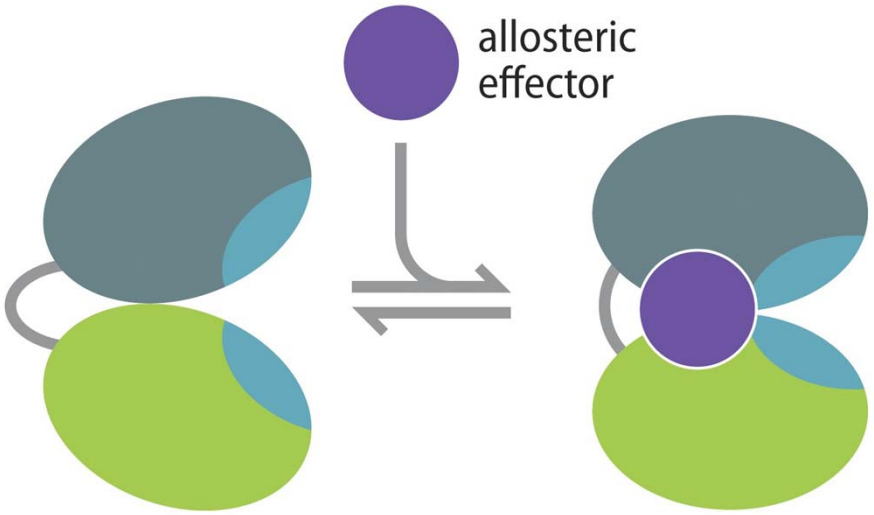



Figure 3.21 How Proteins Work (©2012 Garland Science)

## Bacterial two-component signalling system

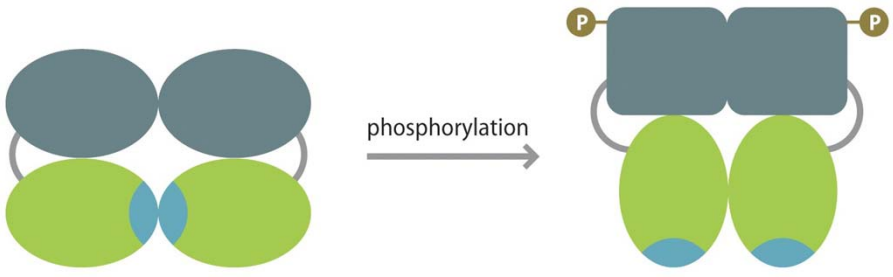
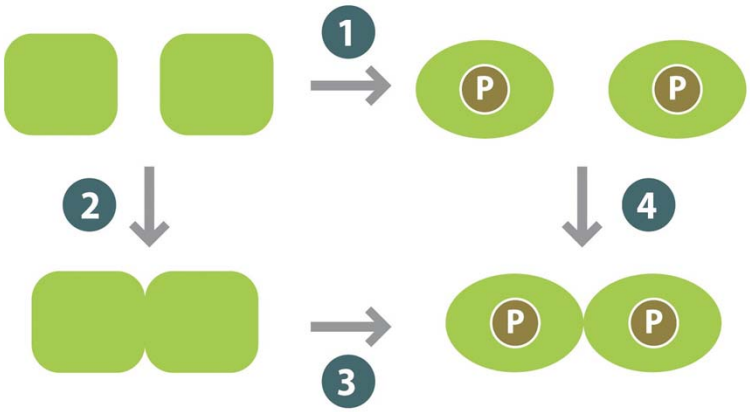



Figure 3.22 How Proteins Work (©2012 Garland Science)

Phosphorylation of the receiver domain (top) leads to a change in conformation, which leads to dissociation of the effector domain (bottom). This domain is then able to dimerize and bind to DNA.

## Simple model for the activation of a dimeric protein by phosphorylation




- Shown as a thermodynamic cycle
- The dimeric phosphorylated protein can then go on to bind to DNA.

## Binding of a protein to a dimer-binding site on DNA

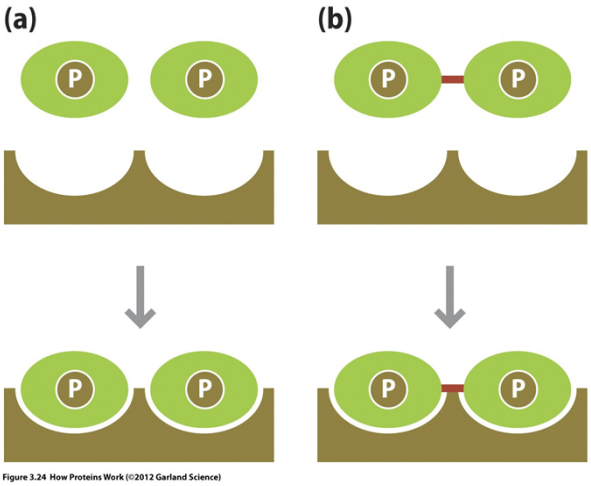



Figure 3.24 How Proteins Work (©2012 Garland Science)

(a) The protein binds as two independent monomers.
(b) The protein is already associated together as a dimer before it binds.

## Probability of occurrence of short DNA sequences

Assuming that DNA sequences have the four bases occuring at random, a given DNA sequence of length $n$ occurs with a probability of $p = 4^{-n}$.

| Length | Probability | Occurences in a genome with $10^7$ bases |
|---|---|---|
| 3 | 0.0156 | $1.6 \times 10^5$ |
| 4 | 0.0039 | $4 \times 10^4$ |
| 6 | $2.4 \times 10^{-4}$ | 2500 |
| 8 | $1.5 \times 10^{-5}$ | 150 |
| 10 | $9.5 \times 10^{-7}$ | 10 |
| 12 | $6.0 \times 10^{-8}$ | <1 |

## Protein binding sites in the major groove of B-DNA

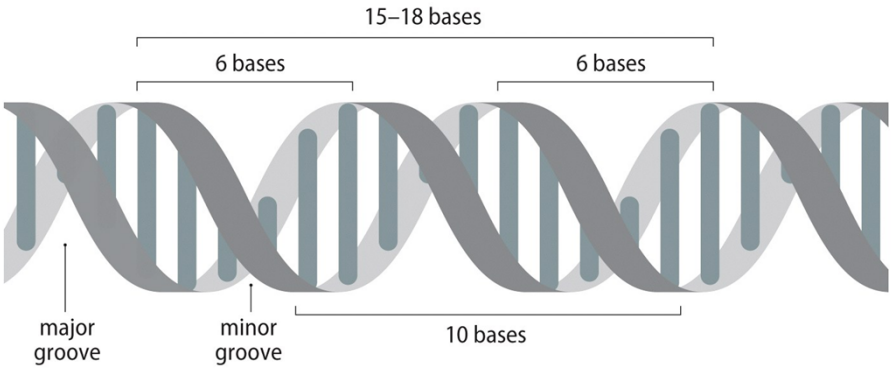



Figure 3.25 How Proteins Work (©2012 Garland Science)

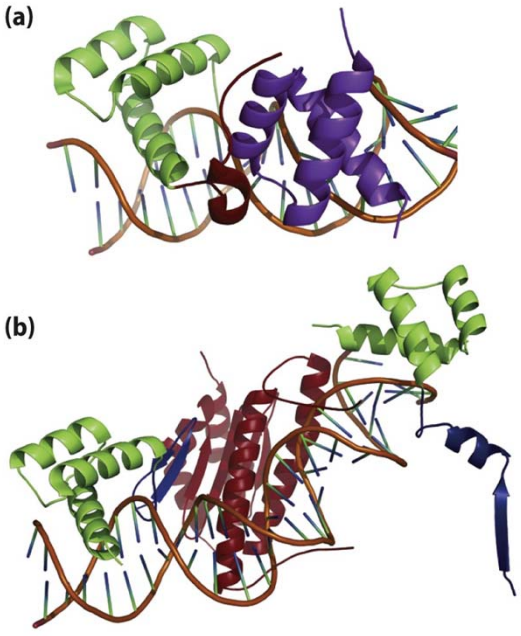
A protein that binds to the major groove of B-DNA and has to come from one face and bind at least 12 bases in total must bind to two groups of 6 bases, and therefore has to span a total of 15–18 bases.

# DNA binding by a dimeric protein


Figure 3.26 How Proteins Work (©2012 Garland Science)

- Nonspecific binding is weak and therefore the protein often dissociates on one side or the other. This weakens the attraction between protein and DNA further, and allows the protein to scan rapidly along the DNA.
- Binding to a specific sequence is stronger and therefore both monomers within the dimer bind to DNA simultaneously.

# Binding of yeast mating factor MAT α2 to DNA




(a) Binding as a heterodimer
(b) Binding as a heterotetramer

Figure 3.27 How Proteins Work (©2012 Garland Science)

## Palindromic DNA binding site for the yeast transcription factor GCN4

5' ATGACGTCAT 3'
3' TACTGCAGTA 5'

Figure 3.28 How Proteins Work (©2012 Garland Science)

- Because in most cases of a homodimer protein binding to DNA the protein dimer has rotational symmetry, so does the DNA that it recognizes.
- Palindromic: The sequence on one strand is the same as the sequence on the other strand read in the opposite direction.

## Helix-turn-helix repressor: *lac* repressor headpiece

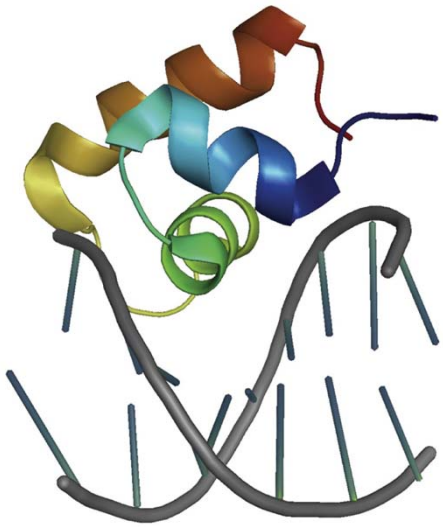
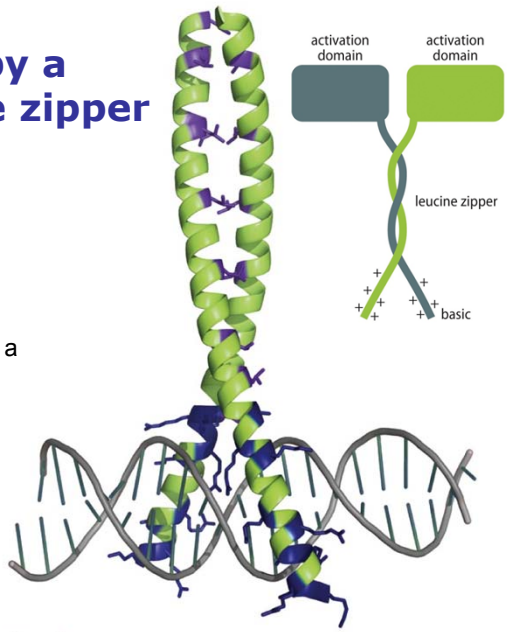


Figure 3.29 How Proteins Work (©2012 Garland Science)

- Recognition helix (green)
- There is a recognition „code“ for sequence-specific recognition by this motif, but it is still not robust enough to be able to predict the DNA sequence from the protein sequence.

## DNA recognition by a symmetric leucine zipper

- Fos-Jun AP1 dimer
- Coiled coil: two α helices wound around each other
- Standard α helix: 3.6 aa/turn
- Coiled coil: 3.5 aa/turn
  → 7 aa for two turns
- Heptad (7-residue) repeats, with a Leu every 7 residues
- Leu from the two helices sit side by side and form a hydrophobic line up the interface





## Summary: Oligomers

- Oligomerization is abundant: Approximately two-thirds of human enzymes are oligomers. In *E. Coli*, the average oligomerization state of proteins is 4.
- In an oligomer, the active site is almost always in a cleft between two domains, which protects it allows access to the active site to be opened and closed easily, and makes allosteric effects easier.
- Oligomerization provides a much larger binding surface and therefore provides increased cooperativity, especially in binding to DNA.
- Almost all allosteric enzymes are oligomers, mainly because an allosteric effector can bind close to the oligomeric interface, and therefore allosteric effects are relatively simple to evolve in an oligomer.

## Literatur

- M. Williamson, *How Proteins Work*, Garland, 2012